

Claims

1. A method for determining endoglycosidase enzyme activity in a sample, comprising the following steps:

- 5 i. bringing a substrate which can be cleaved by an endoglycosidase into contact with said sample, and
- ii. measuring the change in the amount of intact substrate, a decrease in amount of this substrate being representative of endoglycosidase activity in the sample,
- 10 characterized in that the substrate is directly or indirectly labeled with a first donor compound and with a second acceptor compound, and in that the amount of intact substrate is determined by measuring a signal emitted by the acceptor compound, this signal resulting from a transfer, via a close proximity effect, between the donor and the acceptor.

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2. The method as claimed in claim 1, characterized in that the first donor compound and the second acceptor compound are fluorescent compounds, in that the close proximity transfer is an energy transfer, and in that the signal emitted is a fluorescent signal.

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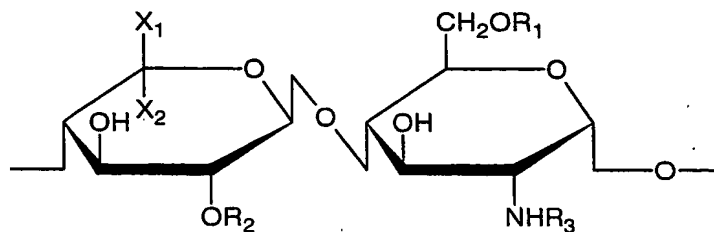
3. A method for detecting a compound capable of modulating an endoglycosidase enzyme activity, comprising the following steps:

- i. bringing a substrate which can be cleaved by an endoglycosidase into contact with an endoglycosidase, in the presence or absence of the test compound,
- 25 ii. measuring the change in the amount of intact substrate, and
- iii. comparing the change in the amount of substrate measured in the absence of the test product with that measured in the presence of the test product,
- 30 characterized in that the substrate is directly or indirectly labeled with a first donor compound and with a second acceptor compound, and in that the amount of intact substrate is determined by measuring a signal emitted by the acceptor compound, this signal resulting from a transfer, via a close proximity effect, between the donor and the acceptor.

4. The method as claimed in claim 3, characterized in that the first donor compound and the second acceptor compound are fluorescent compounds, in that the close proximity transfer is an energy transfer, and in that the signal emitted is a fluorescent signal.

5. The method as claimed in claims 1 to 4, characterized in that the endoglycosidase is an enzyme of the heparanase type chosen from recombinant heparanase, purified heparanase, nonpurified heparanase and heparitinase.

6. The method as claimed in claims 1 to 5, characterized in that the substrate is chosen from heparan sulfate proteoglycans and their derivatives, extracellular matrix-associated heparan sulfates and their derivatives, heparin, and heparan sulfates or their derivatives, and will contain from 1 to 30 units of formula:



in which

R_1 and R_3 are chosen from the groups: H, SO_3H , SO_3H^- ,

R_2 is chosen from the groups SO_3H , SO_3H^- , $\text{C}(\text{O})\text{CH}_3$,

X_1 and X_2 represent H or COOH .

7. The method as claimed in claim 6, characterized in that the substrate is covalently attached to a donor fluorescent compound and to an acceptor fluorescent compound.

8. The method as claimed in claim 6, characterized in that the substrate is covalently attached to a member of a first ligand-receptor pair and to a member of a second ligand-receptor pair, and in that the donor fluorescent compound is covalently attached to the other member of the first ligand-receptor pair and the donor fluorescent compound is attached to the other member of the second ligand-receptor pair.

9. The method as claimed in claim 6, characterized in that the substrate is covalently attached to the donor fluorescent compound and is covalently attached to a member of a ligand-receptor pair, and in that the acceptor fluorescent compound is covalently attached to the other member of said ligand-receptor pair.

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10. The method as claimed in claim 6, characterized in that the substrate is covalently attached to the acceptor fluorescent compound and is covalently attached to a member of a ligand-receptor pair, and in that the donor fluorescent compound is covalently attached to the other member of said ligand-receptor pair.

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11. The method as claimed in claims 8 to 11, characterized in that the first and the second ligand-receptor pair are different and are chosen from the pairs: hapten/antibody, DNP/anti-DNP antibody, GST/anti-GST antibody, biotin/avidin, 6HIS/anti-6HIS antibody; Cmyc/anti-Cmyc antibody; FLAG®/anti-FLAG® antibody; HA/anti-HA antibody.

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12. The method as claimed in claims 1 to 11, characterized in that the donor compound is a rare earth cryptate or chelate, and in that the acceptor fluorescent compound is chosen from rhodamines, cyanins, squaraines, bodipy dyes, fluoresceins, allophycocyanin and their derivatives.

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13. The method for detecting a compound capable of modulating enzyme activity of the heparanase type as claimed in claims 3 and 4, characterized in that said compound is chosen from anti-heparanase antibodies, natural products, synthetic products, products from a library of compounds obtained by combinatorial chemistry, peptides and proteins.

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14. A composition which can be used in one of the methods as claimed in claims 1 to 13, comprising a plurality of HSs comprising biotin and DNP groups, characterized in that the DNP/HS final molar ratio is between 0.3 and 2, and is preferably equal to 0.7, and in that the biotin/HS final molar ratio is between 0.5 and 2, and is preferably equal to 1.

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15. A composition which can be used in one of the methods as claimed in claims 1 to 13, comprising a plurality of HSPGs comprising biotin and DNP groups, characterized in that the DNP/HSPG final molar ratio is between 6 and 15, and is preferably equal to 10.8, and in that the biotin/HSPG final molar ratio is between 6 and 15, and is preferably equal to 8.

16. A kit for carrying out the methods as claimed in claims 1 to 13, comprising the following elements:

- 10 - a substrate which can be cleaved by an enzyme having activity of the heparanase type,
- a donor fluorescent compound covalently attached or capable of indirectly attaching to said substrate,
- an acceptor compound covalently attached or capable of indirectly attaching to said substrate,
- 15 said elements possibly being in the same bottle or in different bottles when the fluorescent compounds are not covalently attached to said substrate.

17. The kit as claimed in claim 16, characterized in that it contains the following elements:

- 20 - a heparan sulfate covalently attached to biotin and to DNP
- a rare earth cryptate covalently attached to an anti-DNP antibody
- a crosslinked allophycocyanin covalently attached to streptavidin.

18. The kit as claimed in claim 16, characterized in that it contains the following elements:

- 25 - a heparan sulfate proteoglycan labeled with biotin and with DNP
- a rare earth cryptate coupled to an anti-DNP antibody
- a crosslinked allophycocyanin coupled to streptavidin.